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CHANGES IN THE LEVELS OF LPO AND GSH IN SWISS ALBINO MICE LIVER AFTER ADMINISTRATION OF ACUTE DOSE OF CYFLUTHRIN (SYNTHETIC PYRETHROID –SOLFAC 050EW)

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ABSTRACT

Given the widespread use of insecticides in the environment, it is important to perform studies evaluating their potential effects on non target species. The use of pyrethroids in household pest control is increasing. Cyfluthrin is extensively used synthetic pyrethroid pesticide. The present study was designed to analyze the liver response to the acute dose of this synthetic pyrethroid in Swiss Albino Mice. The experiments were conducted to determine the concentration of GSH and LPO in the liver of Mice after the administration of acute dose. The value of LPO significantly increased ($p>0.05$) whereas GSH values showed increase followed by significant decline.

KEYWORDS: Acute Toxicity, Cyfluthrin, Synthetic pyrethroid,GSH, LPO

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INTRODUCTION

In developing countries, such as Sri Lanka ,India, pesticide poisonings from short-term very high-level of exposure (acute poisoning) is the most worrisome type of poisoning. However in developed countries, such as Canada, it is the complete opposite: acute pesticide poisoning is controlled, thus making the main issue long-term low-level exposure (1). Farmers depend heavily on synthetic pesticides to control insects(cutworms, ants, silverfish, cockroaches, termites, grain beetles, weevils, mosquitoes, fleas, flies, corn earworms, tobacco budworm, codling moth, European corn borer, cabbageworm, loopers, armyworms etc.), in their crops. Today, it is one of the most commonly used methods for controlling insects. One such synthetic pyrethroids is Cyfluthrin ,which is a synthetic analogues of naturally occurring pesticides, flower of C*hrysanthemum* (3).It was developed to capture the effective insecticidal activity of this botanical insecticide, with increased stability in light, yielding longest residence times (2). This insecticide was first registered for the restricted use (RUP) and general use. It is extensively used to kill unwanted insects in agriculture, in or around buildings, and ornamental plants (4).

Cyfluthrin containing products is classified by EPA as acute Toxicity Category II (bearing the signal word “Warning”) or Toxicity Category I (bearing the signal word “Danger”) based on its potential to cause damage (5). Currently, minimum expected limit of tolerance for residues of cyfluthrin in or on raw agricultural products ranging from 0.05 (hog meat) to 4.0 ppm (hops) (6). Cyfluthrin has been reported to exhibit selective toxicity to insects, with sparingly low toxicity in mammals due to rapid liver metabolism and urinary excretion of both the metabolized and principal compound. However, laboratory animal exposed to relatively high doses of cyfluthrin had shown the same toxic effect observed in insects; including convulsion, salivation, ataxia, weakness and apathy (7,8). Also, report on dermal toxicity has been reported for skin contact (9). Large doses of cyfluthrin cause excess salivation, irritability, tremors, in coordination, convulsions, and a fall in blood pressure .It caused changes in wide variety of organs such as glands, liver, adrenal, spleen and ovary in rats. Liver plays central role in the detoxification of toxicants, there is a tendency for its accumulation and subsequent toxicity to the liver, disrupting the normal hepatic function. Watanable (10) reported liver necrosis and increased chromatin in the nuclei of the hepatic cells after the administration of cyfluthrin.

Environmental agents, such as pesticides, initiate free radical generation that causes different complications in the body (11). The free radicals generated by several metabolic reactions have unpaired electrons on outer orbital (12,13). These radicals are very reactive species and may cause tissue damage and even cell death (12-14). The occurrence of free radicals may increase in certain pathological conditions leading to deleterious effects on several critical molecular and cellular components such as proteins, DNA, and membrane lipids (14). The primary targets of ROS(free radicals) are cell-membrane polyunsaturated fatty acids, which, in turn, lead to damage in the cell structure and function (15). Recent findings have shown that in cases of toxicity, which subsequently decreases cellular energy levels, free radicals are generated within a short period of time (16). These free radicals which are highly reactive molecules cause massive lipid peroxidation (17).

It has been shown that lipid peroxidation have serious effect on some vital functions such as fluidity and selective permeability of membranes as well as signal transduction (17). Moreover, the decomposition of lipid hydroperoxides leads to a wide variety of end products, one of which is malondialdehyde (MDA), which is now accepted as a reliable marker of lipid peroxidation (18).

There are some antioxidant mechanisms

against free radical damage. There are two antioxidant mechanisms namely enzymatic antioxidants such as superoxide dismutase, glutathione peroxidase and catalase, and nonenzymatic antioxidants such as vitamin E (Vit E; á-tocopherol), ascorbic acid and â-carotene as well as reduced glutathione (GSH) and uric acid (17, 18,19). GSH is a cofactor for glutathione peroxidase, which catalyzes the reduction of hydrogen peroxide to water and oxygen, hence limiting the formation of hydroxyl radical, the highly toxic reactive oxygen species (20). GSH is the most abundant intracellular thiol-based antioxidant, prevalent in millimolar concentrations in all living aerobic cells, and plays an important role in the cellular defense cascade against oxidative injury (21, 22). GSH serves to detoxify some endogenic and exogenic compounds with conjugation reactions catalyzed by glutathione S-transferases (20-22).

MATERIALS AND METHODS

Swiss Albino male mice were housed in an air cooled room and a colony was maintained. Mice were fed on standard mice feed (mixed seeds and pellets) and water was given *ad libitum*. For all the present studies adult male mice (4-6 weeks old) were used.

Animals were divided into two groups

Group I (Control): Animals were given distilled water as vehicle orally.

Group II (Acute): Animals were given high dose dissolved in distilled water orally and was given once. The dose administered to the animals was calculated according to the concentration of solfac recommended (8 ml in 1000 litre) for use in field sprays, which came out to be 1.6 μl in 100 μl of distilled water (double of the recommended dose). Autopsy was conducted after 3 hrs, 24 hrs, and 15 days after the dose administration. After the termination of each of experimentation, the treated and control males were sacrificed by cervical dislocation and the liver was perfused carefully with 0.84 % cold saline. Quantitative biochemical estimation of LPO and GSH content at each autopsy interval was conducted. These parameters are sharp indicators of any type of injury or damage in liver cells.

Lipid per oxidation (LPO)

Lipid peroxidation was estimated by the method as described by Ohkawa *et al* (18).

Reduced glutathione (GSH)

The hepatic level of reduce glutathione (GSH) was determined by the method as described by Moron *et al.* (23)

RESULTS

Significant increases in LPO level occurs after the 3 hrs of administration of acute dose as compared to control. The level of LPO decreases after 24 hours and again increases significantly at 15 days. (Table 1).This pattern is indicative of induced toxicity.

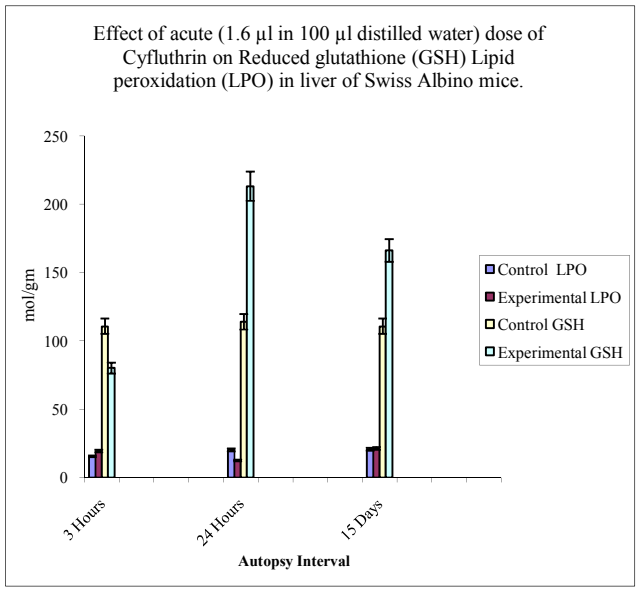
Table 1

Effect of acute (1.6 µl in 100 µl distilled water) dose of Cyfluthrin on Reduced glutathione (GSH) and Lipid peroxidation (LPO) in liver of Swiss Albino mice.

Autopsy Interval	LPO (mol/gm of tissue)		GSH (mol/gm of tissue)	
	Control	Experimental	Control	Experimental
3 Hours	15.6 ±0.54	19.44 ±0.004***	110.72 ± 13.19	80.28 ± 0.36*
24 Hours	20.36 ±3.608	12.49±7.23***	114.06±3.60	213.26 ± 13.72***
15 Days	20.72±10.76	21.37±0.722**	110.72±10.76	166.26 ± 0.61***

Significance in relation to control,* p<0.05,** p<0.01, p<0.001***

The level of GSH shows exactly the reverse trends to the LPO. There is significant decline by p<0.05 in GSH at 3 hours. There is a steep increase in experimental group as compared to control at 24 hours which decline significantly on day 15. (Table 1)



DISCUSSION & CONCLUSION

The depletion in total antioxidant capacity as well as the increment in MDA (lipid peroxidation marker) could be explained by Banerjee et al., (24) who suggested that the formation of oxygen free radical can be a major factor in the toxicity of pesticides. On the other hand, Nasutiet al (25) and Prsanthi et al., (26) reported that oxidative damage, induced by pyrethroids might be due to their lipophilieity whereby they could penetrate easily to the cell membrane and caused membrane lipid peroxidation .Oxidative stress that occurs in the cells, as a consequence of an inequity between the prooxidant/antioxidant systems, causes injury to biomolecules such as nucleic acids, proteins, structural carbohydrates, and lipids [27]. Among these targets, the peroxidation of lipids is basically damaging because the formation of lipid peroxidation products leads to spread of free radical reactions [28]. Peroxidation of lipids can disturb the assembly of the membrane, causing changes in fluidity and permeability, alterations of ion transport and inhibition of metabolic processes [29]. Injury to mitochondria induced by lipid peroxidation can direct to further ROS generation [30]. Lipid peroxidation is one of the major outcomes of free radical-mediated injury to tissue. Peroxidation of fatty acyl groups occurs mostly in membrane phospholipids. Peroxidation of lipids can greatly alter the physicochemical properties of membrane lipid bilayers, resulting in severe cellular dysfunction. In addition, a variety of lipid byproducts are produced as a consequence of lipid peroxidation , some of which can exert adverse and/or beneficial biological effects. Free radical mediated oxidative stress was evidenced by the increased levels of LPO. The accumulation of lipid peroxides introduces hydrophilic moieties into the membrane hydrophobic phase and thus alters membrane permeability and cell functions (31).A cell can tolerate a mild oxidative stress but a higher disturbance between the production of free radicals and the antioxidant action results in membrane damage and corresponding pathological consequences (32). Glutathione peroxidase catalyzes the breakdown of inorganic and organic peroxides and prevents lipid peroxidation and protects the cell membrane from oxidative damage (33). Reduced glutathione has several functions such as a substrate for GSH-Px and glutathione- S-transferase, as well as a direct antioxidant, independent of the enzymes protecting the cell membrane from oxidative damage (23). There is substantial decrease in the level of reduced glutathione (GSH) with concomitant rise in the level of lipid peroxidation in the liver of mice. With acute dose administration, there is initial increase at 24hrs in glutathione (GSH) which probably resulted from an attempt by the organism to control or reverse the biochemical lesion.(34) Reduced levels of GSH confirm an increased susceptibility to oxidative damage and this observation is an agreement with the reports that inverse relationship exists between LPO and glutathione status (35). Glutathione depletion of 20% to 30% can impair the cell against the toxic action of xenobiotics and may lead to cell injury/death.(36,37) It has been reported that decrease in LPO by GSH shows that GSH status in the cell is an important factor to reduce peroxidation(38). The increase in GSH level reveals be recovery of hepatic cells from oxidative stress induced by acute dose of cyfluthrin .As gluthathione is the major cellular nucleophile ,it provides an efficient detoxification pathway (39)for a variety of electrophilic reactive metabolites(40). It has been observed that depressed GSH accompanies increased hepatic lipid peroxidation levels in human beings (37) The initial decrease at 3 hrs, in the glutathione

level observed may be due to increased utilisation by the hepatocytes, because GSH seems to act as scanvenger for toxic chemical agents. Glutathione protects hepatocytes by uniting with reactive metabolites, and thus prevents them from binding covalently to liver proteins. Intracellular decrease of the reduced GSH exposes the cell to the destructive effects of the oxidative stress (41).

There are reports which reveal that there is increase in lipid peroxidation after acute doses of toxicants, which is a result of the consumption in liver glutathione levels (42). Indeed, significant increases in liver lipid peroxidation levels caused a significant decrease in liver glutathione levels; thus glutathione levels play therefore a crucial role in stimulation of lipid peroxidation (43, 44). The results suggest acute dose of Cyfluthrin exhibit hepatotoxicity by increasing free radical generation. The observed abnormalities in the liver is probably due to the alteration in membrane property and function, changes in the activity of anti peroxidative enzymes and GSH and increased LPO. Changes occuring in the activities of antiperoxidative enzymes may therefore expose the cell to the destructive effects of oxidative stress(45).

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